

AIR FORCE OFFICE OF SCIENTIFIC RESEARCH

Grant 90-0222

"Phase-Shifting Effect of Light and Exercise on the Human Circadian Clock"

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STATEMENT OF WORK

In animals, the major environmental signal responsible for the entrainment of circadian rhythmicity to external time is the light-dark cycle. While for many years, it was thought that light did not play an important role in synchronizing human rhythms, and that social cues were the primary entraining agent, extensive evidence obtained during the past decade using light of greater intensity than in earlier studies has indicated that the light-dark cycle is also a major zeitgeber for human circadian rhythmicity. The evolution of concepts regarding zeitgebers for non-human mammalian rhythms ran in many ways opposite to that occurring in the field of human rhythms. Indeed, social and/or behavioral cues were long thought to be ineffective as zeitgebers in rodents and other mammals, but evidence has accumulated over the past few years to indicate that behavioral changes are indeed capable of inducing shifts in circadian rhythms. Specifically, stimuli which cause an alteration of the rest-activity cycle, either by eliciting activity (i.e. locomotor activity in rodent studies) during the normal rest period or by preventing activity during the normal active period, result in phase shifts of circadian rhythms. These findings implied that physical activity during the usual rest period (i.e. nighttime) as well as sleep occurring during the normal active period (i.e. daytime) could alter the phase of human rhythms.

Under the auspices of the present grant, we have developed a novel procedure to determine in humans the immediate phase-shifting effects of light and exercise with the overall goal of obtaining a phase-response curve to photic and non-photic zeitgebers. We are also comparing the effectiveness of manipulation of the light-dark cycle and manipulations of sleep in accelerating the adaptation to shifts of the sleep-wake cycle such as those occurring in "jet lag" and shift work.

STATUS OF RESEARCH EFFORT

1. Overview and adjustments to protocol

At the end of the first year of the grant period, we had completed a series of experiments involving exposure to a 3-hour pulse of bright light or exercise at the time of the minimum of the body temperature rhythm. This is the time when the largest phase-advances are expected to occur in response to light exposure, based on extrapolations from the animal literature as well as on studies using repeated exposure to the light stimulus. Data on the activity, temperature and cortisol rhythms were analyzed. The assays of TSH and melatonin were under progress. Because possible alterations in the timing of the temperature nadir could not be estimated due to the masking effects of recovery sleep, the only markers of circadian phase that was available at the end of the first year were those derived from the cortisol rhythm. Based on the cortisol rhythm, we concluded that light and exercise administered during the later part of the night both

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advance the circadian clock by approximately one hour. We noted that these conclusions were only preliminary, as they needed to be confirmed by the analysis of the TSH and melatonin profiles. During the second year of the grant period, we have completed the measurement of the TSH profiles and have obtained the melatonin profiles in 6 of the 8 subjects who entered the first set of experiments. In addition, we analyzed all the data on behavioral measures, i.e. results on the Stanford Sleepiness Scale, the Digit Symbol Substitution Test and the Symbol Copying Test. As will be shown below, the TSH results confirmed the conclusions derived from the analysis of the cortisol rhythm, but the melatonin profiles failed to indicate the existence of shifts following exposure to either light or exercise. Because the probability levels for significance of shifts in both the cortisol and the TSH profiles were around 0.05, i.e. borderline of traditional limits for statistical significance, we decided to run three additional subjects in this protocol, i.e. with the timing of exposure to the stimulus centered around the minimum of body temperature. We also decided to re-run a selected subset of the samples for the measurement of melatonin in another assay, involving a different antibody than that used by the Takahashi laboratory. Indeed, we expect that any report showing a dissociation between the melatonin rhythm, on the one hand, and the rhythms of cortisol and TSH, on the other hand, would be first scrutinized for the reliability of the melatonin assay, which is notoriously subject to a variety of artefacts. The additional subjects have been run and the re-assay of melatonin is under progress. The need to perform these additional studies has delayed our plans for publication. During the second year of the grant period, we have also run six additional volunteers using an earlier time of exposure to the stimulus (three hours prior to the timing of the minimum of body temperature), which should correspond to delay, rather than advance, shifts. Thus, in relation to the first specific aim of our proposal, during the second year of the grant period, we have examined the magnitude and direction of immediate phase-shifts associated with a single exposure to bright light or physical activity in 9 volunteers, who each participated in three 5-day inpatient studies (baseline, light, exercise). Hormonal assays and statistical analyses for these 27 studies are in progress. Taken together with the work performed during the first year, we have now obtained a 17-point phase-response curve to the immediate response to exposure to a single pulse of light or exercise. The ongoing detailed analysis of these results should answer the following basic questions regarding the human circadian system 1. can the human circadian clock phase-shift in response to a single exposure to light ? ; 2. is exercise a zeitgeber for human rhythms ?

The second specific aim of our proposal consisted of examining the magnitude and direction of stable phase-shifts associated with a single exposure to a pulse of light or physical activity. The analysis of the available results regarding the first specific aim indicates however that the phase-shifting effects of single exposure to light or exercise are of the order of one hour on the first day after the stimulus. Since stable phase-shifts that would still be observed on the third day after exposure to the stimulus would be of smaller magnitude than those observed immediately after stimulus exposure, it is not clear that they could be reliably detected, even with our multiple marker approach. Therefore, to avoid implementing a very difficult, costly and time consuming protocol which may not provide definite answers, we have taken a different approach to further examine the relative power of photic and non-photoc stimuli for human rhythms. This approach consists of examining the adaptation to an 8-hour advance of the sleep-wake and light-dark cycles in subjects treated either with exposure to bright light or with administration of a hypnotic agent which facilitates sleep at a time of normal activity.

Extensive studies in rodents have indeed indicated that exposure to exercise at the time of normal rest (as is the case in our first protocol) or exposure to rest at a time of normal activity are both effective in phase-shifting hamster circadian rhythms. In this second protocol, each subject participates in three studies, one with dim light during waking hours and no hypnotic to facilitate sleep following the 8-hour advance of bedtimes, one with bright light during waking hours and no hypnotic, one with dim light during waking hours and administration of a hypnotic to facilitate sleep at a time of usual activity. During the past year, we have entered six subjects in this new protocol. We expect to have terminated the experimental part of this work by the end of the third year of the grant period.

2. Progress on experimental work

a. *Phase-response curve to single exposure to a 3-hour pulse of light or exercise*

During this second year of the grant period, we have studied 9 normal non-obese men, ages 20-30 years. They were all in good physical condition, none was smoking or taking any drug, none had a personal history of psychiatric or endocrine illness, and all had regular life habits. For each subject, the individual VO_2 max for both legs and arm exercises was determined in the University of Chicago Cardiac Physiology Laboratory using the same exerciser (Schwinn Airdyne) as used during the study. Each study included 5 days of outpatient monitoring and 5 days of inpatient monitoring in the University of Chicago Clinical Research Center. Thus, a total of 135 (i.e. $9 \times 3 \times 5$) outpatient study days and 135 inpatient study days were performed. In three subjects, the stimulus (i.e. bright light of 5,000 lux intensity or exercise) was centered on the time of occurrence of the minimum of body temperature observed under baseline conditions (this occurred at 6:15 in two of the subjects and at 6:00 in the third subject). In the six other subjects, the mid-point of the stimulus was three hours before the minimum of body temperature (range: 00:00-02:00). For exercise, leg and arm exercise were alternated in 5 cycles of 36 minutes each, alternating cycles with high and low workloads (starting with the high workload), and including each 15 min of arm exercise, 15 min of leg exercise and 6 min of rest.

During the 5 days of ambulatory monitoring of wrist activity, the volunteers complied with a standard schedule of bedtimes and mealtimes. The volunteers were then admitted to the CRC and equipped with an ambulatory temperature monitor. Hourly measures of sleepiness on the Stanford Sleepiness Scale and of cognitive performance were taken every hour. In addition to the symbol copying test and digit substitution test which were used during the first series of experiments, we have added two computerized tests which run on a Macintosh system and measure reaction time to an auditory stimulus and relative coordination over a 1-min period. All other details of the protocol were as described in the Annual Technical Report for the first year of the grant period.

Measurements on blood samples include cortisol levels, TSH levels and melatonin levels. We will not monitor glucose, C-peptide and insulin concentrations in this second series of studies since the results from the first series indicated that the circadian modulation of these blood constituents is, under "constant routine" conditions, of too low amplitude to provide accurate markers.

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b. Adaptation to 8-hour advance with bright light or pharmacological sleep induction

In this protocol, each subject participates in three studies spaced at least one month apart. Each study includes two nights of habituation and one baseline 24-hour study with blood sampling at 20-min intervals. Bedtimes are 2300-0700. During the two days immediately following this baseline study, the subjects are submitted to an 8-hour phase advance, i.e. the sleep times are advanced to 1500-2300 and waking/ambulatory conditions are maintained at all other times. During bedtimes, the subjects will remain recumbent in total darkness. During the baseline day as well as during the two following days, sleep is recorded and blood samples for the measurement of cortisol, TSH and melatonin are collected at 20-min intervals. Moreover, body temperature and wrist activity are continuously monitored. In the control study, the subjects will be exposed to light intensities of approximately 200 lux during waking periods. In one of the other studies, the subjects will be exposed to light intensities of 2,000 lux minimum during waking hours. In the third study, the subjects will be exposed to light intensities of 200 lux during waking hours and a hypnotic agent will be administered 15 min before bedtime to facilitate sleep. The order of the three experiments will be randomized. So far, six subjects have entered this protocol and completed at least one of the three studies.

3. Preliminary Data

Table 1 summarizes the results on the TSH profiles from the eight subjects who were studied during the first year of the grant period. As may be seen, the timing of the nocturnal TSH rise was advanced by on average 43 min following exposure to light and 65 min following exposure to exercise. Thus, these data support the concept that a single exposure to a 3-hour pulse of either light or exercise may advance the human circadian clock.

Figure 1 shows the results on the melatonin profiles for 6 of the 8 subjects who were studied during the first year of the grant period. The individual data were expressed in percent of the mean level before calculating the mean for the group, as we observed a considerable inter-individual variation in daytime as well as nighttime melatonin concentrations. Note that exposure to bright light was indeed associated with a rapid decrease in melatonin levels. As mentioned earlier, these samples are being re-assayed using an entirely different melatonin assay. In contrast to the cortisol and TSH results, these data suggest that exposure to a single 3-hour pulse of bright light or exercise has no immediate effect on circadian phase.

Figure 2A, 2B, 2C show the mean profiles for hourly scores on the Stanford Sleepiness Scale (SSS), and hourly results on the Symbol Copying Test and Digit Symbol Substitution Test. for the baseline studies, the studies with light exposure, and the studies with exposure to exercise. In general, the curves for the Digit Symbol Substitution Test and for the Symbol Copying Test are the mirror image of the profile of SSS scores. Note that exercise was associated with a significant decrease in SSS score, resulting in a flatter overall profile. Thus, physical activation during the normal times of sleep was associated with mental activation as well. Exposure to light tended to decrease daytime sleepiness.

PUBLICATIONS (since filing of 01 year report)

Van Cauter E, Linkowski P, Mendlewicz J. Chronobiologie et Psychiatrie. In: Acquisitions en Psychiatrie Biologique, J. Mendlewicz (ed), Masson (Paris, France), 1992, in press.

Van Cauter E, Mestrez F, Sturis J, Polonsky KS. Estimation of insulin secretion rates from C-peptide levels: comparison of individual and standard kinetic parameters for C-peptide clearance. Diabetes 41:368-377, 1992.

Van Cauter E, Kerkhofs M, Caufriez A, Van Onderbergen A, Thorner MO, Copinschi G. A quantitative estimation of GH secretion in normal man: reproducibility and relation to sleep and time of day. J Clin Endocrinol Metab, in press, 1992.

Van Cauter E, Caufriez A, Kerkhofs M, Van Onderbergen A, Thorner MO, Copinschi G. Sleep, awakenings and insulin-like growth factor I modulate the growth hormone secretory response to growth hormone-releasing hormone. J Clin Endocrinol Metab, in press, 1992.

Van Cauter E, Shapiro ET, Tillil H, Polonsky KS. Circadian modulation of glucose and insulin responses to meals: relationship to cortisol rhythm. Am J Physiol. (Endocrinology/Metabolism), in press, 1992.

Van Cauter E. Hormonal and metabolic changes during sleep. Proceedings of the Workshop on "Target Receptors for Anxiolytics and Hypnotics: from Molecular Pharmacology to Therapeutics" organized by the International Academy for Biomedical and Drug Research, Karger, Basel, in press, 1992.

Linkowski P, Van Onderbergen A, Kerkhofs M, Bosson D, Mendlewicz J, Van Cauter E. A twin study of the circadian and pulsatile variations of plasma cortisol: evidence for genetic control of the human circadian clock. Am J Physiol (Endocrinology/Metabolism), accepted pending revisions, 1992.

In preparation

Van Reeth O, Blackman JD, Van Cauter E
Phase-shifting effects of light and exercise in normal man.
to be submitted to Journal of Biological Rhythms, 1992.

Blackman JD, Van Reeth O, Van Cauter E
Circadian modulation of glucose regulation and body temperature during constant conditions of activity, posture and caloric intake.
to be submitted to American Journal of Physiology, 1992

Van Reeth O, Oliner CM, Blackman JD, Polonsky KS, Van Cauter E.
Hormonal effects of exercise during constant glucose infusion.
to be submitted to Journal of Clinical Endocrinology and Metabolism, 1992

Van Cauter E, Turek FW, Van Onderbergen A, Bosson D, Szyper M, Copinschi G. Effects of benzodiazepine treatment on adaptation of human circadian rhythms to an 8-hour delay shift. to be submitted to Am J Physiol, 1992.

PROFESSIONAL PERSONNEL

Blackman John D, M.D.

Fellow in Endocrinology, funded by Endocrinology Training Program at the University of Chicago, will graduate from the Fellowship program on June 30, 1991.

Sturis, Jeppe, Ph.D.

Research Associate, 50% effort from March 1, 1991 to July 1, 1992.

Byrne, Maria, M.D.

Fellow in Endocrinology, funded by Endocrinology Training Program at the University of Chicago, will graduate from program in June 1993. Started in July 1991.

INTERACTIONS

1. Communications at international meetings

Van Cauter E., Invited Speaker, Gordon Conference on Chronobiology, Irsee, Germany, September 29-October 4, 1991.

Van Cauter, E. Effects of sleep on glucose regulation. Invited Speaker. Founding Congress of the World Federation of Sleep Societies, Cannes, France, September 1991, Sleep Research 20A, p 200.

Van Cauter, E. Hormonal and Metabolic changes during sleep. Invited Speaker. Workshop on "Target receptors for anxiolytics and hypnotics: from molecular pharmacology to therapeutics", International Academy for Biomedical and Drug Research, Monte-Carlo, November 1991.

Chairman and organizer of the Symposium on "Human Phase-Shifting and Entrainment", Third Meeting of the Society for Research on Biological Rhythms, Jacksonville, FL, May 1992.

2. Consultative and advisory functions by the Principal Investigator

Invited talk, Enseignement Interuniversitaire Post-Gradué d'Endocrinologie (University of Brussels, University of Liège, University of Louvain), Brussels, December 7, 1991.

Invited talk, Département de Médecine, Hôpital Tivoli, La Louvière, Belgium, February 4, 1992 (Dr. Ducobu).

Invited talk, Laboratoire de Psychologie et de Physiologie Environnementales, CNRS, Strasbourg, France, February 7, 1992 (Dr. G. Brandenberger).

TABLE 1

TIMING OF NOCTURNAL TSH RISE

	DAY 1 PRE STIMULUS	DAY 2 POST STIMULUS	
BASAL	21:35 \pm 20 min	21:17 \pm 28 min	NS
3-H LIGHT PULSE	21:13 \pm 25 min	20:30 \pm 29 min	p= .06
3-H EXERCISE PULSE	22:00 \pm 30 min	20:55 \pm 14 min	p=. 05

FIGURE 1

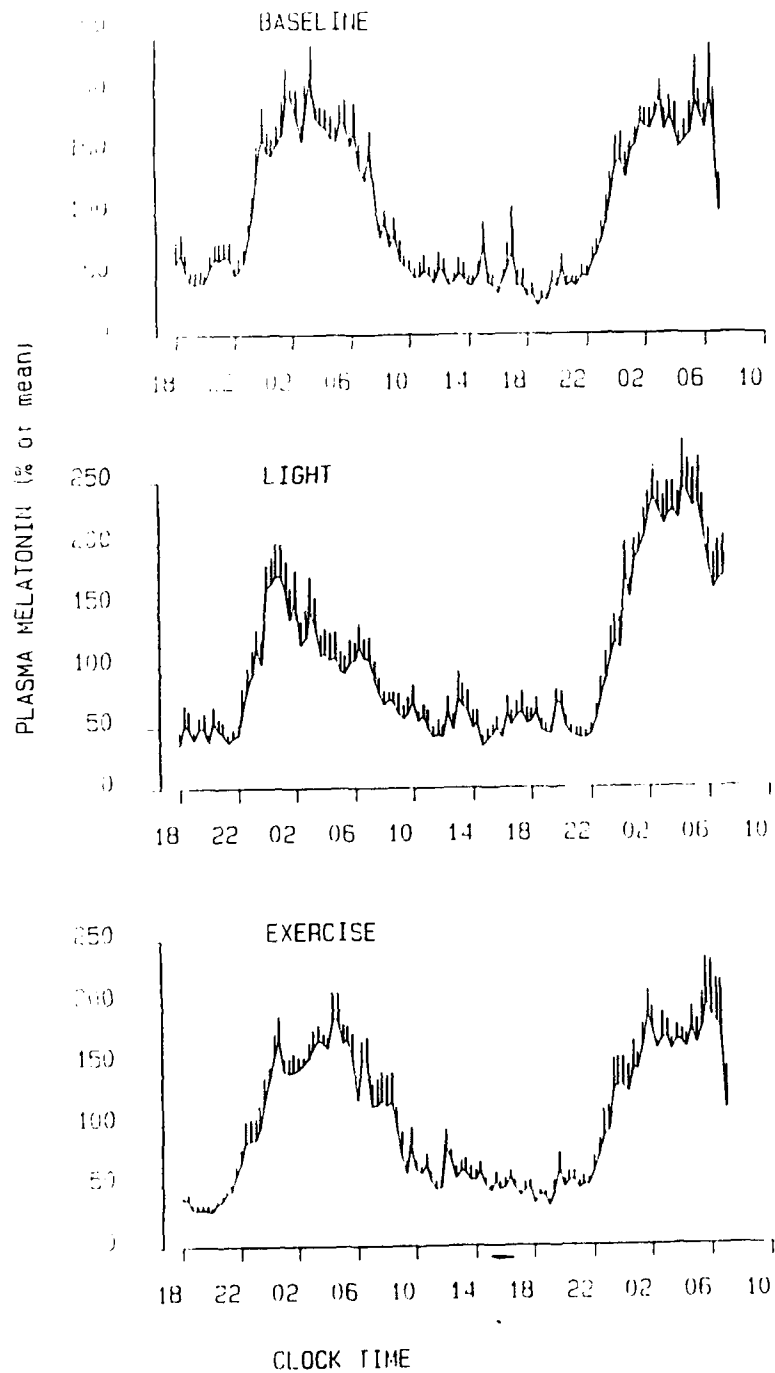


FIGURE 2A Baseline studies

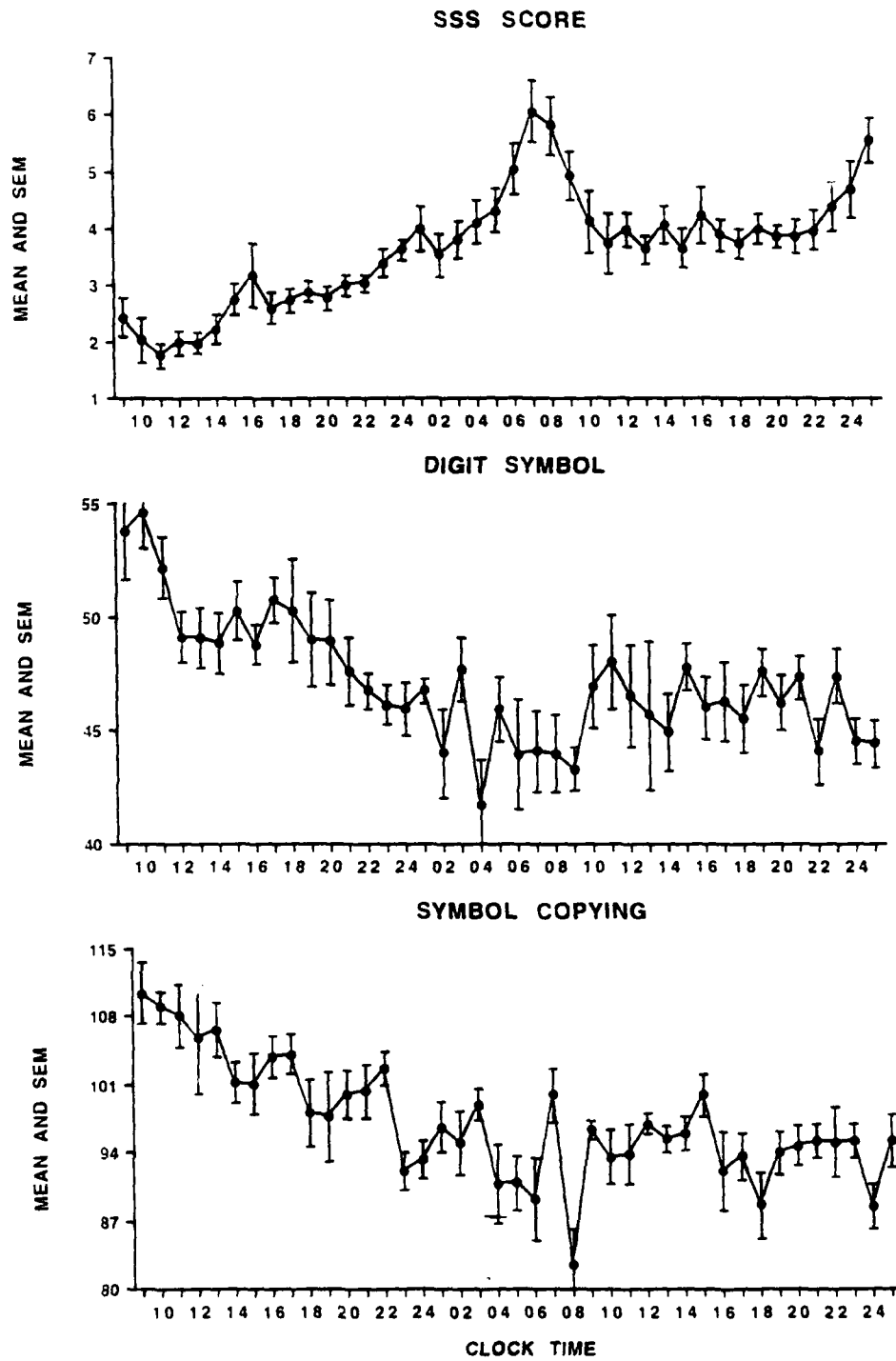


FIGURE 2B Light exposure

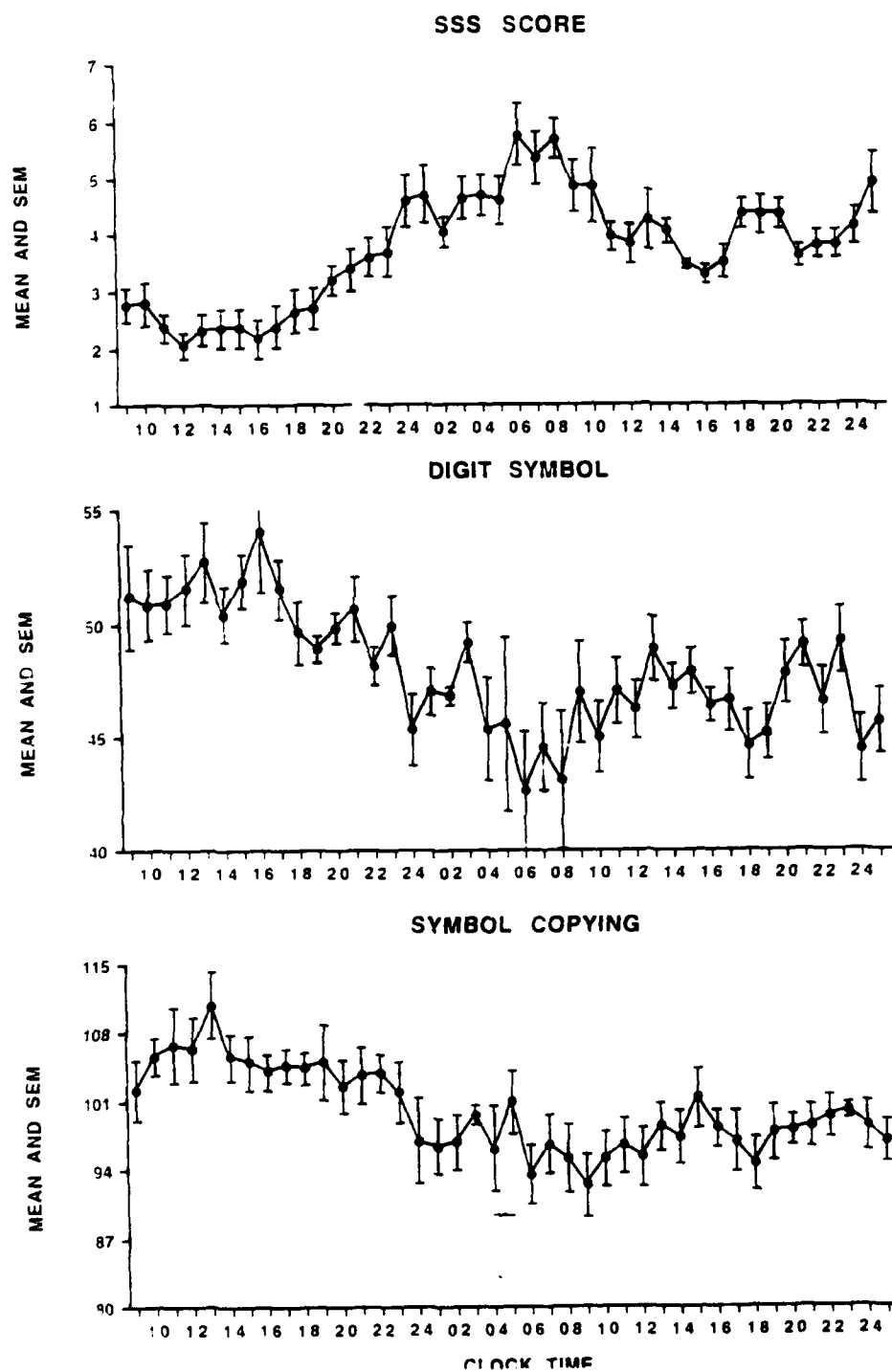


FIGURE 2C Exercise exposure

